A Prototype Antifungal Contact Lens

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Purpose. To design a contact lens to treat and prevent fungal ocular infections.

Methods. Curved contact lenses were created by encapsulating econazole-impregnated poly(lactic-co-glycolic) acid (PLGA) films in poly(hydroxyethyl methacrylate) (pHEMA) by ultraviolet photopolymerization. Release studies were conducted in phosphate-buffered saline at 37°C with continuous shaking. The contact lenses and their release media were tested in an antifungal assay against *Candida albicans*. Cross sections of the pre- and postrelease contact lenses were characterized by scanning electron microscopy and by Raman spectroscopy.

Results. Econazole-eluting contact lenses provided extended antifungal activity against *Candida albicans* fungi. Fungicidal activity varied in duration and effectiveness depending on the mass of the econazole-PLGA film encapsulated in the contact lens.

Conclusions. An econazole-eluting contact lens could be used as a treatment for fungal ocular infections. (*Invest Ophthalmol Vis Sci.* 2011;52:6286–6291) DOI:10.1167/iovs.10-6935

Fungi are a leading cause of ocular infection in tropical and subtropical climates, which cover much of the developing world,[1,2] and also in temperate climes.[2,3] The prevalence of fungal keratitis continues to increase in industrialized countries, particularly after trauma or surgery.[4] Topical ophthalmic solutions, or eye drops, are the most common treatment for fungal keratitis, but 15% to 27% of patients require surgical intervention.[5] The failures of topical antimycotic treatments may be related to the limitations of eye drops as a form of drug delivery. Eye drops generate a transiently high concentration on application followed by a short period of effective therapeutic concentration and then a prolonged period of underdose. Furthermore, each drop is diluted and washed away by reflex tearing and dispersed by blinking. As a consequence, only 1% to 7% of drug in a drop is absorbed into the eye.[6] The cornea absorbs only a fraction of this dose, in part due to the tissue's short contact time with the topical drops.[7]

Other factors prevent antifungal drops from reaching fungicidal levels in the cornea. At low concentrations, the drugs may be fungistatic, stopping proliferation of the organisms within the tissue. However, to kill fungi, higher drug concentrations are necessary.[8] Furthermore, many drugs have difficulty penetrating the corneal epithelium.[9–11] The barrier effect can be overcome with higher drug concentrations and prolonged contact time with the tissue.[10] Consequently, patients with fungal keratitis frequently need to self-administer antifungal drops every hour[12,13]—initially day and night.[11] If the treatment regimen is effective, the drop frequency is reduced, but the regimen persists for weeks to months.[11] Not surprisingly, compliance can be problematic, both acutely and over long periods. A sustained-release system for ophthalmic drugs, including antifungals, could improve compliance and treatment efficacy.

The concept of delivering drugs specifically through a contact lens was introduced as early as 1960.[14] Several researchers designed contact lenses for drug delivery,[15,16] but no products are currently commercially available. Recently, we have described a contact lens composed of a drug-loaded polymer film contained within poly(2-hydroxyethyl methacrylate) (pHEMA), a polymer commonly used in the manufacture of contact lenses.[17] With a flat prototype contact lens, a near zero-order release of relatively large quantities of drug was achieved over at least 30 days for both the fluorescent indicator sodium fluorescein and the antibiotic ciprofloxacin.[17]

Here, we describe the formulation and characterization of a contact lens designed for extended release of an antifungal drug, econazole. Econazole was chosen for its broad-spectrum antifungal activity,[18] low cost, and wide availability in the developing world. It has been shown to be effective in the treatment of fungal keratitis.[8] Poly(lactic-co-glycolic) acid (PLGA) was used to fabricate the econazole-polymer films encapsulated inside the contact lenses. PLGA is well-known for its biocompatibility, biodegradability, and ability to control drug-release kinetics.[19–22]

Materials and Methods

High-molecular-mass (118 kDa) PLGA (65:35; 65% lactic acid and 35% glycolic acid) was obtained from Lakeshore Biomaterials (Birmingham, AL). A photoinitiator (Irgacure 2959) was purchased from Ciba Specialty Chemicals Corporation (Tarrytown, NY). Econazole powder, 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), glucose, histidine, leucine, and all the other reagents were purchased from Sigma-Aldrich (St. Louis, MO). *Candida albicans* strain SC5314 was regrown from frozen stocks from the Whitehead Institute (Boston, MA). Phosphate-buffered saline (PBS, pH 7.4) was obtained from Invitrogen (Carlsbad, CA). Yeast nitrogen base (YNB)

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and yeast extract peptone (YEP) media from DIFCO (BD Biosciences, Franklin Lakes, NJ) were used.

Solvent-Casting of Drug-Polymer Films

To create the drug-polymer film, 100 mg of PLGA was dissolved in 4 mL of ethyl acetate. Econazole (200 mg) was added to the solution and vortexed to form a fine suspension, which was poured into a Teflon well. Ethyl acetate was removed by evaporation in a fume hood with laminar air flow overnight. The resulting films were then lyophilized for 48 hours. Films with three different surface areas (12.8, 55.4, and 180 mm^2) were punched out of the econazole-PLGA film. PLGA-only films, created using the same process, using 100 mg of PLGA and 4 mL of ethyl acetate, but lacking econazole, served as the control.

Coating the Drug-Polymer Film in pHEMA

All films were coated with pHEMA by ultraviolet (UV) photopolymerization.[17] A HEMA solution was mixed and degassed as previously described.[17] The solution was then transferred into a contact lens mold (14 mm in diameter and 150 μm in height) and placed in a nitrogen atmosphere. The monomer solution was polymerized with a 305-nm UV lamp over 60 minutes to form the bottom pHEMA layer of the composite contact lens. The dried pHEMA gel was then transferred into a deeper contact lens mold (14 mm in diameter and 500 μm in height). After the drug-PLGA film and PLGA film (control) were manually pressed onto the dried pHEMA gel, an additional 150 μL of the monomer/photoinitiator solution was added to the mold and UV-photopolymerized under the same conditions. The resulting contact lens prototype consisted of a thin drug-PLGA film encapsulated on all sides with pHEMA. Each lens was assigned a code PLGAx, where *x* was the total number of milligrams of econazole contained within the contact lens (Table 1).

Econazole Antifungal Assay

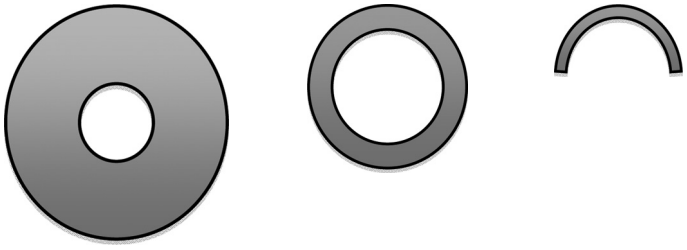
Growth of *C. albicans.* *C. albicans* strain SC5314 was regrown from frozen stocks from the Whitehead Institute. In all cases, *C. albicans* was maintained on YEP-agar plates. For experiments, liquid YEP (50 mL) was inoculated with one colony overnight. After that time, the suspension was centrifuged (5 minutes at 8000*g*), the supernatant was discarded, and the cells were washed twice with PBS (pH 7.2; 50 mL). The cells were counted on a hemocytometer and diluted to 0.5 to 1×10^7 or 2 to 4×10^7 cells/mL in YNB medium with 50 mM glucose.

Fungicidal Effect of Econazole. To test the fungicidal effect of econazole, the drug was suspended in 225 μL of YNB medium over a range of concentrations (0–1 mg/mL). An additional 75 μL of fresh YNB containing 2 to 4×10^7 cells/mL *C. albicans* SC5314 was subsequently added. After 2 hours, the suspension was diluted by a factor of 1000, 200 μL of the diluted suspension was plated onto YEP agar plates and incubated at 37°C for 24 hours, after which the viable colonies were counted. The fungicidal effect of econazole against *C. albicans* was assessed in duplicate.

Fungicidal Effect of Direct Exposure to the Contact Lenses. To test the effect of directly exposing the contact lenses to fungi, we immersed the contact lenses in 1 mL of YNB medium with 50 mM glucose and incubated them at 37°C, with shaking at 100 rpm. The YNB medium was replaced every 24 hours with fresh medium. At predetermined intervals, the contact lenses were removed, irrigated with YNB medium, and placed in the wells of a 24-well tissue culture plate with 1 mL of the *Candida* suspension ($0.5–1 \times 10^7$ cells in YNB). The lenses were incubated for 2 hours at 37°C, with shaking at 100 rpm. The contact lenses were subsequently removed, placed in fresh YNB medium with 50 mM glucose, and incubated at 37°C, with 100-rpm shaking. The remaining medium was vigorously stirred and subsequently diluted by a factor of 1000. Next, 200 μL of the diluted medium was plated on YEP agar plates. The YEP plates were incubated at 37°C for 24 hours, and yeast colonies were counted on both the contact lens–exposed samples and a yeast-only control. The fungicidal activity, as calculated here, is in part a result of the experimental design (e.g., starting fungal cell counts). We cannot exclude the possibility that fungal cell killing may have been less than 100% in some samples if cells had been seeded at higher concentrations.

Fungicidal Effect of Drug Release. To assess the fungicidal effect of the econazole-releasing contact lenses, the lenses were immersed in 1 mL of YNB medium with 50 mM glucose, shaken at 100 rpm, and incubated at 37°C. This is the standard temperature for the purpose of testing the extraction from materials and medical devices, including contact lenses.[23–25] Previous studies demonstrated no significant difference between the growth of C. *albicans* at 37°C and 35°C,[26] which has been found to be the surface temperature of the cornea with the lids open.[27,28] After this incubation, the medium was removed completely, and the contact lenses were immersed in fresh medium every 24 hours. YNB drug release medium (225 μL) was collected at each time point and subsequently diluted with 75 μL of fresh YNB containing 2 to 4×10^7 cells/mL *C. albicans* SC5314. After 2 hours, the suspension was diluted by a factor of 1000, plated onto YEP agar plates, and incubated at 37°C for 24 hours, after which viable colonies were counted in the same manner as above. When the YEP recovery plates were examined and the colonies counted after 24 hours (3–4 days), the colony counts remained unchanged beyond the 24-hour period. Therefore, we report the growth after 24 hours of growth. Control samples were prepared by adding 75 μL of YNB containing 2 to 4×10^7 cells/mL *C. albicans* SC5314 to 225 μL of YNB.

Table 1. Morphology, Dimensions, and Amount of Econazole Contained within Each Type of PLGA Film

Film Label	$PLGA_{16}$	$PLGA_4$	$PLGA_1$
Morphology			
Outer diameter, mm	15.9	13.7	11.1
Inner diameter, mm	4.7	9.5	9.5
Surface area, mm^2	180.0	55.5	12.8
Econazole, mg/film	15.5	3.6	0.9

Effect of Freeze-Drying on the Fungicidal Effect of Contact Lenses. A preliminary test was conducted to ascertain the effect of freeze drying on the econazole-loaded contact lenses. A pair of contact lenses containing an econazole-PLGA film ($PLGA_{16}$) was quick frozen with liquid nitrogen and lyophilized for 24 hours. The freeze-dried lenses were subsequently tested for the fungicidal effects associated with direct exposure to the contact lenses, as described above.

Scanning Electron Microscopy

Antifungal contact lenses containing an econazole-PLGA film ($PLGA_{16}$) were immersed in 1 mL of PBS at 37°C, shaking at 100 rpm. The medium was changed every 24 hours for 10 days. The contact lenses were imaged before and after drug release. The dry lenses were cross-sectioned with a microtome and sputter coated with a gold-plutonium alloy under vacuum (Hummer 6.2; Anatech Limited, Union City, CA). Images were acquired with a scanning electron microscope (JSM 5600 LV; JEOL USA. Inc, Peabody, MA).

Raman Spectroscopy

Econazole, a microtome cross-sectioned dry HEMA gel (lacking an econazole-PLGA film), and microtome cross-sectioned lenses (containing an econazole-PLGA film, $PLGA_{16}$) were placed in a Raman spectrometer with microprobe (Hololab 5000R; Kaiser Optical Systems, Inc., Ann Arbor, MI). Raman spectroscopy measurements were performed with a 28-mW solid-state laser (785 nm) with a 100-μm fiber, a 10× objective, and an acquisition time of 120 seconds. For the cross-sectioned lenses, the laser spot was centered in the middle of the drug-polymer film.

Results

Drug-PLGA Films

The econazole-PLGA film was produced through solvent casting, then punched and/or cut into various shapes (Table 1), and lyophilized to remove residual ethyl acetate. After lyophilization, the films measured 150 μm in thickness. Of note, the flexibility of the films (with and without econazole) decreased substantially after lyophilization due to the elimination of residual ethyl acetate.

pHEMA Coating Using Composite Construction

Econazole-PLGA films and PLGA-only films were encapsulated inside a hydrogel layer as previously described.[17] The final contact lens measured 14 mm in diameter and 450 μm in thickness in its dry state. After hydration, the prototype contact lens demonstrated the flexibility typical of soft contact lenses and measured 15.5 mm in diameter, representing an increase of approximately 10% from its dehydrated state. The base curve (radius of an imaginary sphere with the curvature of the back of the contact lens) was 8.05 mm. The central lens remained free of the drug-polymer film after pHEMA encapsulation (Fig. 1, Table 1).

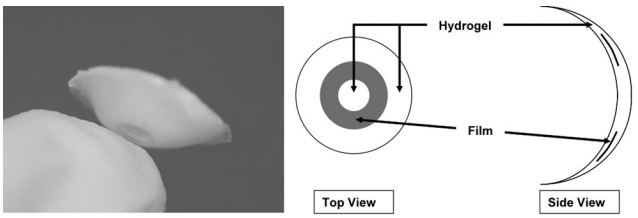


Figure 1. *Left*: Photograph of prototype contact lens containing an econazole-PLGA film with a surface area of 180 mm^2. Note the clear optical axis. *Right*: schematic of prototype antifungal contact lens.

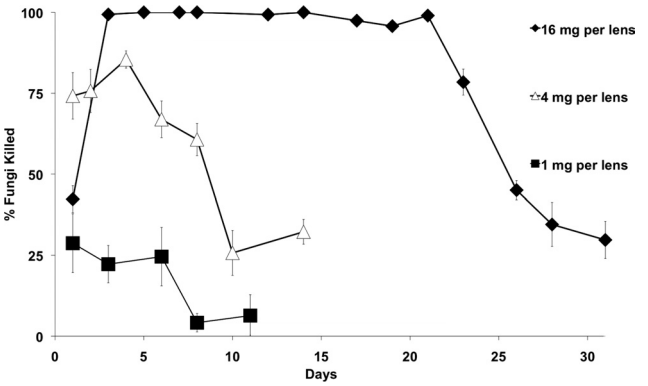


Figure 2. Antifungal activity of YNB media containing econazole eluted from drug-containing contact lenses. Data are the mean ± SD. $n = 4$.

Antifungal Activity

We have developed antifungal devices that can kill fungi by releasing fungicidal activity into surrounding media and/or by direct contact between the antifungal surface and yeast.[29,30] The capacity of released econazole to effectively kill fungi was verified by incubating econazole-loaded contact lenses in YNB fungal medium for predetermined time intervals, at which points the lenses were removed, and the release media were tested for fungicidal activity after 24 hours of growth.

Econazole released from the lenses effectively killed fungi over an extended period (Fig. 2). Release medium from contact lenses containing 16 mg of econazole ($PLGA_{16}$) killed 100% of fungi for 21 days. Release medium from lenses containing lower amounts of econazole were less efficacious and killed for shorter periods.

Previously, we have shown that hydrogels containing amphotericin B have the desirable property of killing fungi rapidly (i.e., complete killing within 2 hours), and can maintain that level of fungicidal activity over long periods.[29,30] To determine whether the drug-eluting contact lens could do the same, lenses were placed in medium containing 1 mL of the *Candida* suspension ($0.5–1 \times 10^7$ cells in YNB) for 2-hour periods at predetermined intervals (Fig. 3). The fungi were completely killed by exposing fungal suspensions directly to $PLGA_{16}$ contact lenses up to 8 to 10 days after the beginning of the drug release process. Lenses with lower econazole loadings killed lower percentages of fungi over approximately the same period, whereas the control lens had no impact on fungal viability and became overgrown within 2 days.

Long-term storage of such lenses could require lyophilization to prevent drug elution and/or degradation of the biodegradable

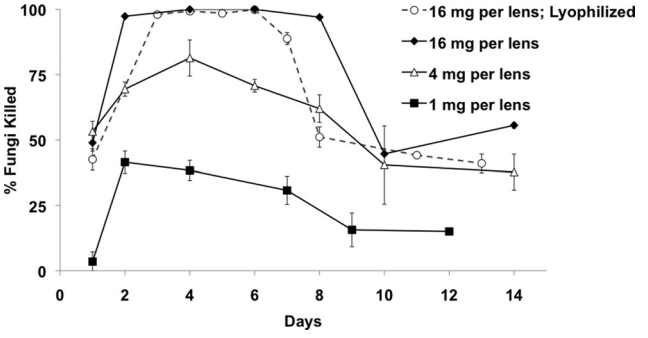


Figure 3. *C. albicans* killed by direct exposure to the econazole-containing contact lenses. Data are the mean ± SD. $n = 4$.

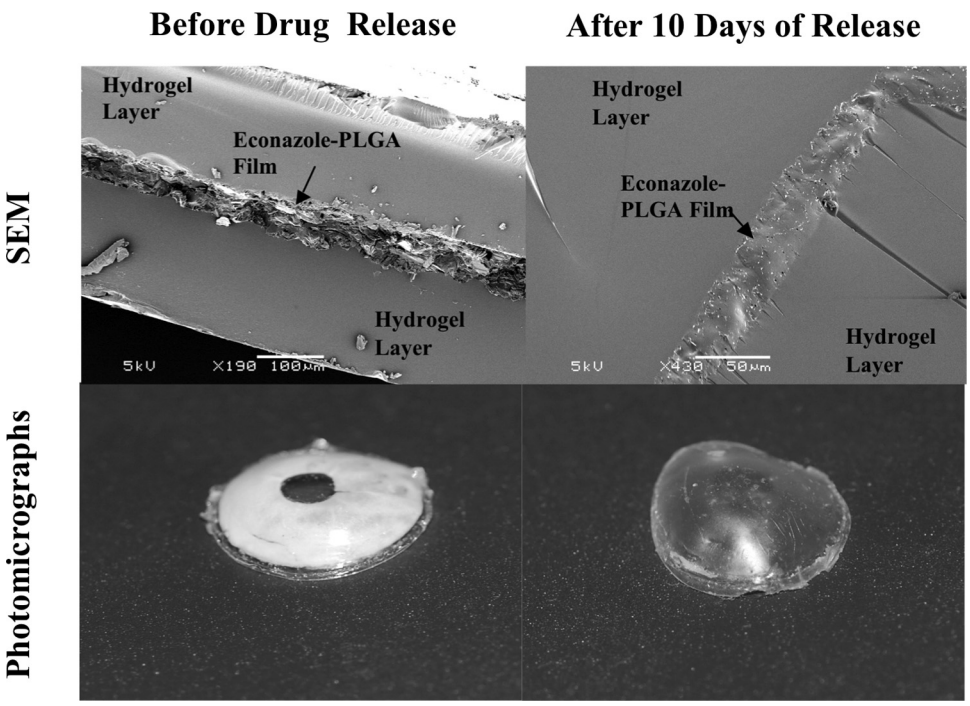


FIGURE 4. Scanning electron and photomicrographs of econazole-PLGA films within pHEMA hydrogels before and after 10 days of drug release. Magnification: *top left*, ×190; *top right*, 430× magnification.

component (if any). To address the effect of such storage, the fungicidal activity of the optimally effective $PLGA_{16}$ contact lens was assessed after 24 hours of lyophilization (Fig. 3). The lyophilized $PLGA_{16}$ lens exhibited fungicidal activity similar to that of the nonlyophilized lens, although the duration of antifungal activity was 1 to 2 days shorter for the lyophilized lens.

Lens Characterization before and after Drug Release

Contact lenses were imaged before and after 10 days of drug release (Fig. 4). When imaged by scanning electron microscopy (SEM), the econazole-PLGA layer appeared as a structurally heterogeneous layer sandwiched between the homogeneous layers of lens hydrogel. After 10 days of drug release, the econazole-PLGA layer contained some small pores and appeared substantially depleted, thinner, and more homogeneous. The SEM images were consistent with the gross appearance of the embedded films in the contact lenses, which appeared significantly less opaque after releasing econazole for 10 days.

To ascertain whether any drug was left in the lenses after 10 days of drug release, Raman spectroscopy was performed on cross-sectioned contact lenses before and after release. A contact lens containing the econazole-PLGA film (before release) demonstrated spectral peaks seen in crystalline econazole (3073, 3061, and 2964 cm^{-1}) and cross-linked HEMA (2941 and 2884 cm^{-1}; Fig. 5, top). After 10 days of drug release, the HEMA peaks (2941 and 2884 cm^{-1}) were still present, but the spectral peaks characteristic of econazole were no longer clearly observed when viewing the Raman shift from 0 to 3300 cm^{-1}. However, on zoom, several minor peaks (423, 391, and 244 cm^{-1}) indicative of econazole were demonstrated on the contact lens after 10 days of release (Fig. 5, bottom), and this may account for the fungicidal effect observed beyond 10 days (Figs. 2, 3).

DISCUSSION

We have produced a curved antifungal contact lens with the ability to kill fungi for at least 3 weeks (Fig. 2). The efficacy and duration of the killing was dependent on the drug loading of the contact lens. Econazole, the drug released in this study, is U. S. Food and Drug Administration (FDA)–approved for topi-

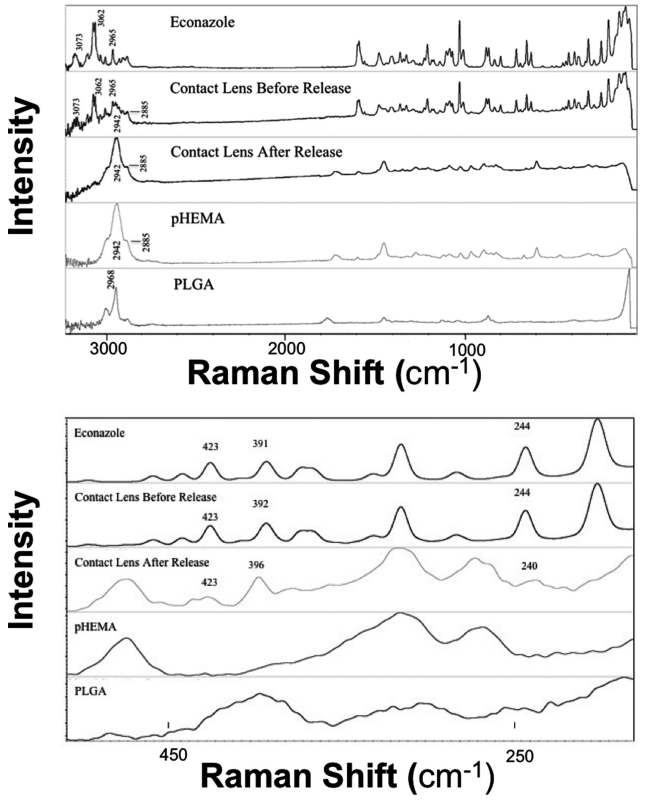


FIGURE 5. Raman spectra of econazole, a pHEMA film, a PLGA film, and a contact lens containing an econazole-PLGA film (PLGA16) before and after 10 days of drug release. *Top*: shift from 0 to 3300 cm^{-1}. Zoom of the Raman spectra (bottom, 180–550 cm^{-1}) demonstrated multiple peaks (423, 391, and 244 cm^{-1}) in the contact lens that were indicative of econazole.

cal use in the treatment of skin infections, but has not been approved for ophthalmic use. In the United States, there is only one drug (natamycin 5%) that is FDA approved for topical ophthalmic use to treat fungal ocular infections.[31,32] From a survey of American ophthalmologists, half would prefer to use more than one medication as a first-line treatment, and most would use a different antifungal than natamycin for monotherapy, if the various antifungals were readily available.[32] Econazole is commonly used in the developing world and in the United Kingdom.[33] We chose it because of its low cost, wide availability in the developing world, and broad-spectrum antifungal properties.[18] It is effective at killing both filamentous fungi and molds. Comparative minimum inhibitory concentration (MIC) and in vivo data suggest that *C. albicans* is more difficult to kill than *Fusarium* when treating with econazole.[34] Therefore, since this device is effective at killing *C. albicans*, then it should also kill *Fusarium*, which has a lower MIC for econazole. Azole antifungal drugs have been used as adjunctive therapies for amebic keratitis; it is conceivable that they could play the same role via a drug-eluting lens.

PLGA, the polymer used here, has been widely studied and FDA approved for ocular use.[19–22] Using a similar contact lens design (drug-polymer film encapsulated in HEMA hydrogel), we have previously demonstrated that the rate of drug release could be altered by either changing the molecular weight of the PLGA or by changing the proportion of PLGA to drug within the film.[17] Based on these observations, it is likely that the duration of fungicidal action could have been lengthened by using a PLGA with a higher molecular mass or by increasing the ratio of PLGA to drug within the film.

The curved nature of the lens studied here is an advance over our previously reported proof-of-principle prototype drug-eluting lens.[17] Both the curvature (8.05 mm base curve) and the diameter (15.5 mm) are consistent with measurements of commercially available lenses.

Storage of drug-eluting lenses could be problematic if in the wet state, particularly if the drug-containing polymer were biodegradable, as it was here. Lyophilization, or freeze drying, is therefore an attractive potential means of storage.[35] Lyophilization did not have an appreciable effect on the ability of the lenses to kill fungi.

An econazole-eluting contact lens would expand ophthalmologists' armamentarium for treating fungal keratitis, which can be difficult to diagnose and to treat. Because fungus may not grow from cultures for up to 14 days, ophthalmologists often initiate treatment based on the clinical scenario and the cornea ulcer's appearance. An inadequate treatment response could be due to several factors that include an inaccurate fungal diagnosis or poor patient compliance. Therefore, poor patient compliance can complicate the ophthalmologist's treatment decision-making process. In the case of fungal keratitis, compliance is particularly difficult because of the taxing nature of the treatment regimen, which initially includes hourly drop administration throughout the night. For the patients who are compliant, the lack of sleep adds to the stress of this potentially blinding condition.

A potential concern is whether sustained elution of econazole or any other antifungal agent would lead to more rapid selection of resistant forms. Although this matter deserves close study, it seems unlikely that the development of resistance would be a greater issue than is the case with the alternative, which is eye drops.

A drug-eluting contact lens may entail considerations relating to lens care. These considerations are minimized if the lens is worn continuously. Similar to a bandage contact lens, a fungicidal contact lens could be inserted by an ophthalmologist and left in place until it is removed and/or replaced by the physician. However, it is important to understand that if the lens is removed by the patient, it will continue to release drug, and any biodegradable components will continue to degrade, albeit at a slower rate if stored at room temperature. The release would continue until some equilibrium was reached with the drug in the storage solution. In the case of an antifungal contact lens, release while in storage could be helpful in preventing fungal keratitis, which has been identified as a problem with lens care.[36]

Based on this study, an econazole-eluting contact lens could effectively kill *Candida* for weeks at a time using an HEMA hydrogel. The hydrogel materials used here were model compounds used for proof-of-concept. It may be possible to use other hydrogel materials, such as a silicone-based hydrogel which is more oxygen-permeable and may be a better candidate for a continuous-wear contact lens. However, it is possible that the design or specific elements of the production process would have to be modified to accommodate a change in the hydrogel material, or that some materials would not be amenable to formulation in the manner described here. Therefore, additional testing would be necessary to determine the feasibility of incorporating a silicone hydrogel into this design.

When developing a medical device for clinical use, it is important to consider the regulatory path that the product must travel. In the United States, a drug-releasing contact lens may be regulated by the FDA as a combination drug device, since it contains more than one regulated component.[37] The primary mode of action of the lens would be determined by the FDA who would then assign the lead center (drug or device) based on this decision.[37] Fungal keratitis would likely be the treatment indication for an econazole-releasing contact lens.

In the industrialized world, an antifungal contact lens could improve the treatment of mycotic eye infections by reducing the treatment burden and by increasing patient compliance. However, warm, humid areas of the developing world may most benefit from this device, since the inhabitants of those regions experience a high prevalence of mycotic eye infections.[1,32,38,39] Because of trachoma and other diseases, they also have a high rate of cornea blindness, but ophthalmologists have been reluctant to use a keratoprosthesis in this patient population because of the fear of fungal endophthalmitis. In such areas, econazole-releasing contact lenses may not only improve the treatment of mycotic eye infections, but they may also expand the surgical options to include keratoprosthesis and other forms of ocular reconstruction.

Conclusion

We have designed a curved, econazole-releasing contact lens that consists of a econazole-PLGA film encapsulated within a pHEMA hydrogel. The contact lenses retained their fungicidal effects over the course of 3 weeks. Such lenses may offer an alternative treatment of fungal keratitis and may also represent a platform for ocular drug delivery with applications beyond infectious eye diseases.

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